

Investigations on some physiological parameters involved in chlorosis occurrence in different grapevine rootstocks and a *Vitis vinifera* cultivar

L. BAVARESCO

Cattedra di Viticoltura, Università Cattolica S. C., Via Emilia Parmense, 84, I-29100 Piacenza, Italy

S u m m a r y: 1-year-old grapevine cuttings were grown in pots in order to test, during the growing period, the changes of some leaf compounds related to chlorosis occurrence (chlorophylls a, b and total chlorophyll, Fe²⁺, macronutrients and trace elements).

The genotypes tested were three rootstocks showing an increasing degree of chlorosis resistance (*Vitis riparia* x *V. rupestris* 101-14, *V. berlandieri* x *V. riparia* SO 4, *V. berlandieri* x *V. rupestris* 140 Ru) and a *V. vinifera* variety (Chardonnay), each of them grown in both a calcareous and a non-calcareous soil.

At the end of the growing period, the whole cuttings were analysed to test the macronutrients and trace elements content of the dry matter.

The most important findings are:

- (1) During the growing period, the chlorophyll and leaf Fe²⁺ content first increases and then decreases.
- (2) The rootstock most susceptible to chlorosis (101-14) shows in the calcareous soil the highest Fe²⁺ and total leaf chlorophyll content, while the most resistant one (140 Ru) has the lowest values. Therefore, the analysis of such parameters is not a suitable tool to screen rootstocks for chlorosis resistance.
- (3) Suitable tools to judge the resistance/susceptibility to lime-induced chlorosis in ungrafted rootstocks grown on calcareous soils are: a) the dry matter production at the end of the annual growing cycle; b) the 'iron efficiency ratio' (g dry matter/mg iron) in the shoot at the end of the annual growing period.

Key words: chlorosis, resistance, rootstock, variety of vine, soil, lime, chlorophyll, iron, mineral, growth.

Introduction

Many world-wide agricultural crops, grown in calcareous soils, suffer from lime-induced chlorosis, usually recognized by yellowed intervein areas in new leaves. Plant species mainly affected include apples, avocado, bananas, barley, beans, citrus, cotton, grapes, oats, peanuts, pecans, potatoes, sorghum, soybeans and numerous greenhouse flowers (CHEN and BARAK 1982). The most important factor responsible for lime-induced chlorosis is the high bicarbonate (HCO₃⁻) concentration in the soil solution (BOXMA 1972; MENGEL and MALISSIOVAS 1981; MENGEL and BÜBL 1983; MENGEL *et al.* 1984; COULOMBE *et al.* 1984; MENGEL and GEURTZEN 1986; KOLESH *et al.* 1987) related to high pH (JUSTE *et al.* 1967). Use of soil Fe by plants is genetically controlled; a variety that can use Fe in an alkaline soil is called Fe-efficient, while a variety that develops iron chlorosis is called Fe-inefficient (BROWN and JONES 1976). Mobilization of iron in the rhizosphere is due to both basic or non-specific mechanisms (independent from the iron nutritional status of the plant) and adaptive mechanisms (MARSCHNER 1986), which are activated in Fe-efficient plants in response to iron-stress. The adaptive mechanisms differ among genotypes and they can be classified according to two strategies (MARSCHNER *et al.* 1986).

Strategy I (exhibited by most higher plants, dicotyledons and monocotyledons except for grasses) consists of four types of response in the roots, as follows: a) enhancement of H⁺-ions release (MARSCHNER 1978; LANDSBERG 1981); b) formation of rhizodermal or hypodermal transfer cells (KRAMER *et al.* 1980; LANDSBERG 1989); c) enhancement of ferric iron reduction to ferrous iron (BIENFAIT *et al.* 1982); d) enhancement of release of reducing/chelating compounds such as phenolics (RÖMHELD and MARSCHNER 1981; HETHER *et al.* 1984).

Strategy II, occurring in barley, oats, rice and probably most other grasses, is characterized by an enhancement of release of non-proteinogenic amino acids (phytosiderophores) and by a high affinity uptake system (RÖMHELD 1987).

Table 1: Physical and chemical characteristics of the soils

	Non-calcareous soil	Calcareous soil
pH in H ₂ O	6.9	8.3
pH in KCl	5.9	7.7
Sand	31%	29%
Silt	45%	55%
Clay	24%	16%
Carbonates	Absent	54%
Lime	Absent	19%
Organic Matter	1.6%	0.3%
CEC	12.9 mEq/100 g	10.1 mEq/100 g
Soluble Salts	210 µS/cm	225 µS/cm
C/N ratio	10.9	8.2
Total nitrogen	0.8%	0.2%
Phosphorus (P ₂ O ₅) 1)	63 ppm	11 ppm
Potassium (K ₂ O) 2)	146 "	71 "
Magnesium 2)	179 "	27 "
Calcium 2)	1960 "	1920 "
Sodium 2)	11 "	9 "
Iron 3)	130 "	89 "
Manganese 3)	225 "	42 "
Zinc 3)	7 "	3 "
Copper 3)	10 "	3 "
Boron 4)	3.4 "	0.3 "

- 1) extracted by Olsen method
- 2) exchangeable cation extracted by NH₄OAc 1N at pH = 7
- 3) extracted by NH₄OAc 0.5 N + EDTA 0.02 M at pH = 4.65
- 4) extracted by Truog method and analysed by Azomethine H

Phytosiderophores are specific Fe chelating compounds such as mugineic and avenic acid (TAKAGI *et al.* 1984; RÖMHELD and MARSCHNER 1986).

The tolerant grapevine rootstocks probably have strategy I response mechanisms (BAVARESCO *et al.* 1989), but vines are normally grafted and the behaviour of the whole plant towards lime-induced chlorosis is governed by two properties: 1. the ability of the roots to supply the iron needs of the leaves; 2. the leaves iron requirement to secure a normal iron nutrition of the plant (POUGET and OTTENWALTER 1973).

In the present work, the ranges of some physiological parameters involved in chlorosis occurrence in ungrafted rootstocks are discussed. It is of further interest to study the reactions of different genotypes, which are known from the field, to affect chlorosis symptoms of the scion with different intensities.

Table 2: Rootstock resistance to chlorosis based on soil IPC (from POUGET and JUSTE 1972; POUGET 1980)

Rootstock	Maximum threshold for IPC 1)
Violla	2
Riparia Gloire	5
3309, 101-14	10
Rupestris du Lot	20
99 R, 110 R, SO4, 1103 F	30
Kober 5BB, 420 A	40
161-49, 41 B	60
333 EM	70
140 Ru	90
Fercal	120

$$1) \text{ IPC} = \frac{\text{CaCO}_3}{(\text{Fe})^2} \cdot 10^4$$

CaCO₃ = active lime (%)

Fe = iron (ppm) extracted by ammonium oxalate

Materials and methods

1-year-old grapevine cuttings (about 10 cm long) rooted in sand were grown in pots in both a non-calcareous and a calcareous soil (Table 1).

The genotypes tested were three rootstocks (related with a decreasing degree of chlorosis resistance in the scion) (Table 2) and a *Vitis vinifera* cultivar, as follows: *V. berlandieri* x *V. rupestris* 140 Ru, *V. berlandieri* x *V. riparia* SO4, *V. riparia* x *V. rupestris* 101-14, Chardonnay clone R 8.

The shoot length was weekly gauged for each genotype in both soils.

15 d after beginning of the trial (1st sampling time), 80 d later (2nd sampling time) and 115 d later (3rd sampling time), the 4th and the 5th leaf (counting from the tip of the shoot) were collected. After washing of the leaves in 1% NaOCl solution, the following constituents were determined:

Fe (II): It was expressed as $\mu\text{g/g}$ dry wt (ppm) and $\mu\text{g/g}$ fresh wt, using the method of KATYAL and SHARMA (1980). 2g of fresh-chopped samples were added to 20 ml of 1.5% 1,10-phenanthroline solution at pH 3 in 100 ml glass bottles. After 16 h, the contents were filtered and Fe(II) was estimated in the filtrate by measuring the absorbency of the Fe(II)-phenanthroline complex at 510 nm.

Chlorophylls: Chlorophyll a, b and total chlorophyll were expressed in mg/100 g d. wt and mg/g f. wt. They were extracted from leaf discs by using 80% acetone for 72 h in the dark, at +4 °C (TORRECILLAS *et al.* 1984). The chlorophyll concentration was determined by reading the absorbencies at 665 nm and 649 nm and use of the equations given by STRAIN and SVEC (1966).

Mineral elements: Macronutrients (N, P, K, Ca, Mg) and some trace elements (B, Fe, Mn, Cu, Zn) were analysed after wet destruction of the dry matter using the methods of COTTENIE (1980).

At the end of the annual growing period, the plants were divided into leaves, shoot, trunk, roots and each part was analysed for dry matter and mineral elements content.

Table 3: Effect of sampling time, genotype and soil on the Fe(II) and chlorophyll contents of leaves

	SAMPLING TIME			GENOTYPE				SOIL				
	1st	2nd	3rd	LSD	140 Ru	101-14 S04	64 Ch	LSD	68 n.c.	64 c.		
Fe ⁺⁺ ppm	68	84	45	3.9	56	61	82	64	4.6	68	64	3.2
Fe ⁺⁺ $\mu\text{g/g}$ fresh wt	17.7	22.8	17.0	1.11	17.2	17.7	21.2	20.7	1.28	19.6	18.8	0.79
Chl. a mg/100 g dry wt	333	491	338	36.3	344	370	423	411	41.9	382	393	NS
Chl. b mg/100 g dry wt	152	243	164	12.1	162	182	203	199	13.9	183	189	NS
Tot. chl. mg/100 g dry wt	499	734	502	39.2	506	551	629	626	45.3	573	583	NS
Chl. a/b dry wt	2.27	2.02	2.06	0.07	2.16	2.06	2.11	2.09	NS	2.11	2.10	NS
Chl. a mg/g fresh wt	0.96	1.43	1.03	0.08	0.93	1.09	1.16	1.37	0.1	1.14	1.14	NS
Chl. b mg/g fresh wt	0.42	0.71	0.50	0.03	0.44	0.54	0.56	0.64	0.04	0.54	0.55	NS
Tot. chl. mg/g fresh wt	1.39	2.14	1.52	0.13	1.37	1.63	1.72	2.01	0.15	1.67	1.69	NS
Chl. a/b fresh wt	2.27	2.02	2.06	0.04	2.16	2.05	2.08	2.16	0.05	2.13	2.10	NS

Ch = Chardonnay ; NS = not significant

n.c. = non calcareous ; c = calcareous

The statistical plan included three-way ANOVA and two-way ANOVA with interactions; the means were compared by LSD test at a 5 % level.

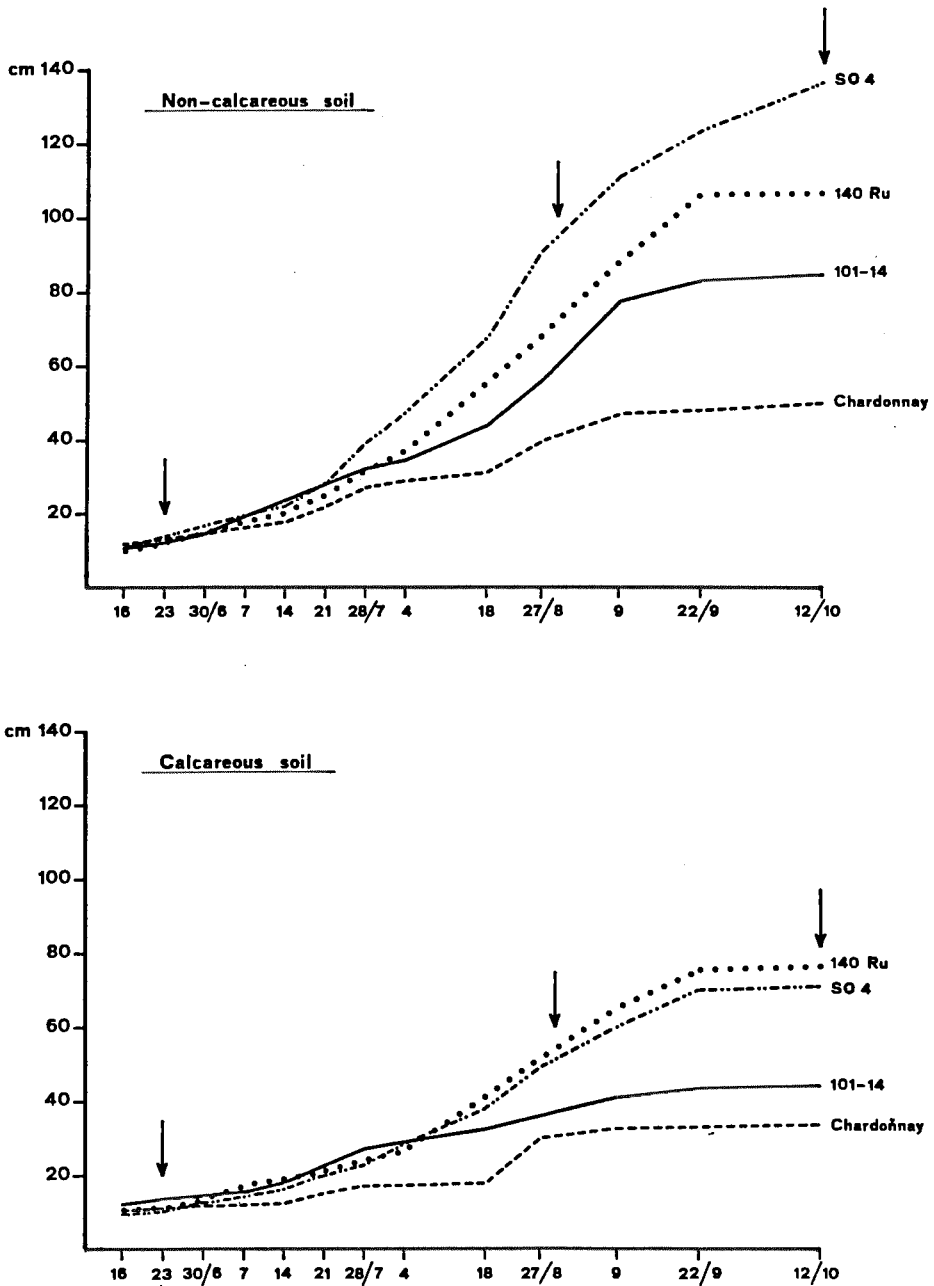


Fig. 1: Shoot growth in the two different soils depending on genotype. Arrows indicate the three sampling times.

Results

The shoot growth (Fig. 1) seems to be affected by both soil and genotype. The calcareous soil has a negative effect on the growth of each genotype.

The highest shoot length in the non-calcareous soil occurs in SO 4 (136 cm), while in the calcareous one 140 Ru grows highest (76 cm). 101-14 has within the rootstocks the lowest shoot growth in both soils.

The Fe(II) content of the leaves (based on both dry and fresh weight) is affected in a significant way by the sampling time, the genotype, the soil and their interactions (Table 3). The values increase from the 1st sampling time (68 ppm and 17.7 $\mu\text{g/g}$ f. wt) to the 2nd one (84 ppm and 22.8 $\mu\text{g/g}$ f. wt) and then decrease at the 3rd sampling time (45 ppm and 17 $\mu\text{g/g}$ f. wt).

101-14 rootstock shows the highest Fe(II) content (82 ppm and 21.2 $\mu\text{g/g}$ f. wt), while 140 Ru has the lowest one (56 ppm and 17.2 $\mu\text{g/g}$ f. wt).

The plants grown on the calcareous soil show a lower iron content (64 ppm and 18.8 $\mu\text{g/g}$ f. wt) than those from the non-calcareous one (68 ppm and 19.6 $\mu\text{g/g}$ f. wt).

The sampling time and the genotype influence the chlorophylls content in a significant way. Total chlorophyll (on the basis of both dry and fresh weight) first increases (changing from 499 mg/100 g d. wt and 1.39 mg/g f. wt at the 1st sampling time to 734 mg/100 g d. wt and 2.14 mg/g f. wt at the 2nd sampling time), then it decreases to 502 mg/100 g d. wt and 1.52 mg/g f. wt.

140 Ru rootstock shows the lowest chlorophyll content (506 mg/100 g d. wt and 1.37 mg/g f. wt); on the other hand, 101-14 shows within the rootstocks the highest value (629 mg/100 g d. wt and 1.72 mg/g f. wt).

The differences due to the two soils are not significant. The total chlorophyll and leaf Fe(II) content, on a fresh weight base, are well related when the plants are grown under stress condition (calcareous soil) (Fig. 2).

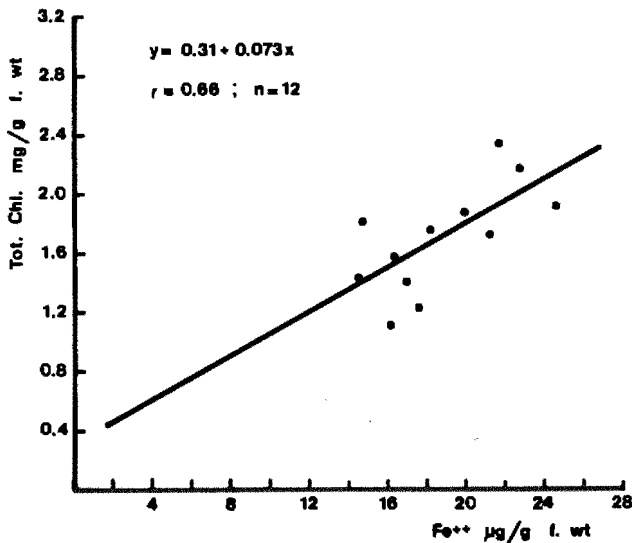


Fig. 2: Correlation between leaf Fe(II) and total chlorophyll content of the genotypes grown on calcareous soil.

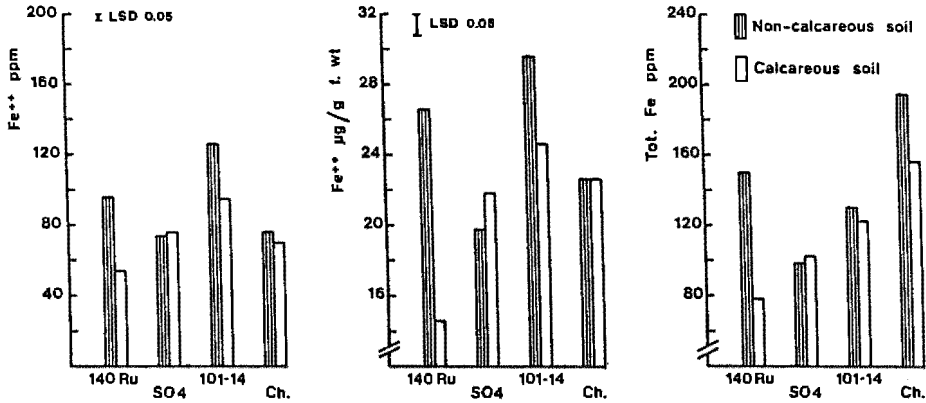


Fig. 3: Leaf ferrous iron and total iron content depending on genotype and soil at the 2nd sampling time.

When focusing the attention to the 2nd sampling time (the period of the fastest shoot growth), it is interesting to observe the behaviour of each genotype as influenced by the soil. The Fe(II) content decreases from the non-calcareous soil to the calcareous soil for each rootstock, save for SO 4 where it increases from 19.7 µg/g f. wt to 21.8 µg/g f. wt (Fig. 3).

In the calcareous soil, the total chlorophyll content changes within the rootstocks from 674 mg/100 g d. wt (101-14) to 742 mg/100 g d. wt (SO 4) (Fig. 4).

The effects of the sampling time, the genotype and the soil on the mineral element content of the leaves are summarized in Table 4. The behaviour of the macronutrients depending on the sampling time is different, while the trace elements have a uniform trend. Nitrogen first increases and then decreases, changing from 2.58 % to 3.20 % and 1.92 %. Leaf potassium content increases (from 0.98 % to 1.28 %), while calcium and magnesium decrease. The trace elements, except Cu, decrease with progress of the growing season.

Among the genotypes, Chardonnay variety shows the highest contents of nitrogen, calcium, manganese and zinc, while 101-14 rootstock has the highest potassium value. The leaf iron content is 155 ppm in 140 Ru, 151 ppm in Chardonnay, 133 ppm in 101-14 and 124 ppm in SO 4.

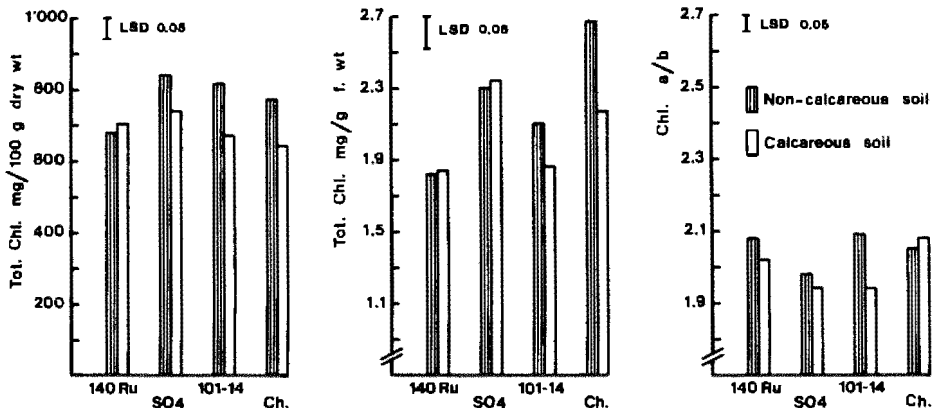


Fig. 4: Leaf total chlorophyll content and chlorophyll a/b ratio depending on genotype and soil at the 2nd sampling time.

Table 4: Effect of sampling time, genotype and soil on the mineral element content of leaves

	SAMPLING TIME			GENOTYPE				SOIL	
	1st	2nd	3rd	140 Ru	S04	101- -14	Ch	n.c.	c.
NZ	2.58	3.20	1.92	2.34	2.34	2.71	2.89	2.61	2.53
PZ	0.23	0.22	0.22	0.21	0.20	0.24	0.24	0.23	0.21
KZ	0.98	1.14	1.28	0.96	1.08	1.31	1.20	1.18	1.09
CaZ	1.30	0.51	0.55	0.78	0.74	0.79	0.84	0.71	0.87
MgZ	0.40	0.34	0.30	0.35	0.32	0.35	0.36	0.33	0.37
Fe ppm	172	129	122	155	124	133	151	144	138
Mn ppm	106	69	53	72	71	71	89	84	67
Cu ppm	23	30	23	29	29	19	24	24	26
Zn ppm	118	39	27	58	59	58	70	55	67
B ppm	17	8	10	12	12	11	11	12	12
P/Fe	13.4	17.0	18.0	13.5	16.1	18.0	15.9	16.0	15.2
Fe/Mn	1.62	1.87	2.30	2.15	1.75	1.87	1.70	1.71	2.06

Ch = Chardonnay

n.c. = non calcareous ; c. = calcareous

The effect of the soil does not seem to be strong, save for calcium, where the plants grown on calcareous soil have a value higher than those grown on the non-calcareous one (0.87 % vs 0.71 %).

At the end of the annual growing period, the organ and the genotype affect the content of all the elements (Table 5), whereas the soil influences in an appreciable way the plant content of Ca (1.00 % and 1.51 % in the non-calcareous and calcareous soil, respectively) and Fe (377 ppm and 186 ppm, correspondingly).

Among the genotypes, the dry matter production is affected by soil above all in 101-14 (Fig. 5), which has in the calcareous soil the lowest value of the rootstocks (3.2 g). Though 101-14 in the calcareous soil has the highest leaf iron content (355 ppm) among the rootstocks (Table 6), it shows the lowest 'iron efficiency ratio' (g dry matter/mg Fe) in the shoot (Fig. 6).

Discussion

The results obtained during the growing period emphasize the role of the shoot growth stage and the genotype on some physiological parameters of the leaf involved in chlorosis occurrence.

Table 5: Percentage of dry matter, total dry matter and mineral element content of the plant depending on organ, genotype and soil at the end of the annual growing period

	ORGAN					GENOTYPE					SOIL		
	Leaves	Shoot	Trunk	Roots	LSD 0.05	140 Ru	S04	101-14	Ch.	LSD 0.05	n.c.	c.	LSD 0.05
Dry matter (%)	27.95	37.06	50.81	35.81	2.63	37.54	36.46	37.80	39.83	2.63	38.45	37.37	NS
Dry matter (g)	4.37	3.53	6.22	4.15	0.59	5.02	5.17	4.56	3.52	0.59	5.25	3.88	0.42
N%	1.67	0.75	0.57	1.15	0.08	0.92	0.94	1.02	1.26	0.08	1.03	1.04	NS
P%	0.17	0.11	0.07	0.17	0.02	0.12	0.12	0.13	0.16	0.02	0.13	0.13	NS
K%	1.16	1.32	0.37	0.81	0.10	0.81	0.90	0.96	0.99	0.10	0.92	0.91	NS
Ca%	2.23	0.89	0.71	1.74	0.12	1.13	1.16	1.11	1.61	0.12	1.00	1.51	0.08
Mg%	0.37	0.15	0.10	0.19	0.02	0.19	0.17	0.19	0.26	0.02	0.19	0.22	0.01
S%	0.17	0.06	0.04	0.14	0.01	0.11	0.09	0.08	0.13	0.01	0.09	0.11	0.01
B ppm	22	18	13	13	1.7	16	17	16	18	1.7	17	17	NS
Fe ppm	330	61	141	594	100	256	238	233	399	100	377	186	71
Mn ppm	73	30	36	26	7	31	32	36	48	7	44	29	5
P/Fe	5.72	21.40	6.32	4.93	2.9	9.75	10.66	9.29	8.67	NS	8.62	10.57	1.94

Ch.= Chardonnay ; n.c. = non-calcareous ; c. = calcareous

Regarding the role of the genotype, 101-14 is of special interest. This rootstock, which normally induces chlorosis in the scion when growing on a calcareous soil, does not show any chlorosis symptom when it is ungrafted. Leaf Fe(II) content of 101-14 is even higher than in the other rootstocks, as well as the chlorophylls. Only at the stage of fastest shoot growth, the total chlorophyll content of 101-14, growing on the calcareous soil, is lower than in the other rootstocks, but without visual differences. This behaviour seems strange, because in trials performed on excised roots 101-14 showed a low reducing capacity and uptake rate for iron (BAVARESCO *et al.* 1989). This rootstock (ungrafted) is probably able to mobilize under stress conditions from the nutrient reserves stored in the cutting a higher iron amount than the other rootstocks, thus supplying the high iron needs of the leaves. This hypothesis is supported by the data of Table 6, where a negative correlation seems to be between leaf and trunk iron of the three rootstocks growing on the calcareous soil. Besides this, not always high iron uptake capacity means high transport inside the plant to the leaves (NERKAR and MISAL 1987).

Table 6: Total iron content (ppm) of the plant depending on organ, genotype and soil at the end of the annual growing period

	LEAVES	SHOOT	TRUNK	ROOTS
	140 SO4 101- Ru -14 Ch.	140 SO4 101- Ru -14 Ch.	140 SO4 101- Ru -14 Ch.	140 SO4 101- Ru -14 Ch.
Non-calcareous soil	234 221 308 501	73 43 49 78	140 141 190 233	1000 833 572 1421
Calcareous soil	240 278 355 502	45 52 47 99	137 108 85 93	178 230 256 265

Ch. = Chardonnay

The lack of yellowing in the leaves (measured in this work by the chlorophyll content) of a rootstock susceptible to chlorosis when growing in a calcareous soil was already observed by POUGET and JUSTE (1972). These authors explained this apparently paradoxical behaviour by the iron requirement of the leaves, which is different in ungrafted and grafted plants. Another explanation considers the negative effect of the grafted vine's yield on the nutrient reserves, including iron, in the woody parts of the plant (BALASUBRAHMANYAM *et al.* 1978); excessive yield induces chlorosis symptoms in the following year (MURISIER and BRIGUET 1988), depending on the reduction of the sugar reserves in the roots (POUGET 1974).

This different behaviour of a grafted and an ungrafted rootstock disappears when a seedling or a softwood cutting is tested instead of a woody cutting (BYRNE 1988; ROMERA *et al.* 1989 a, 1989 b).

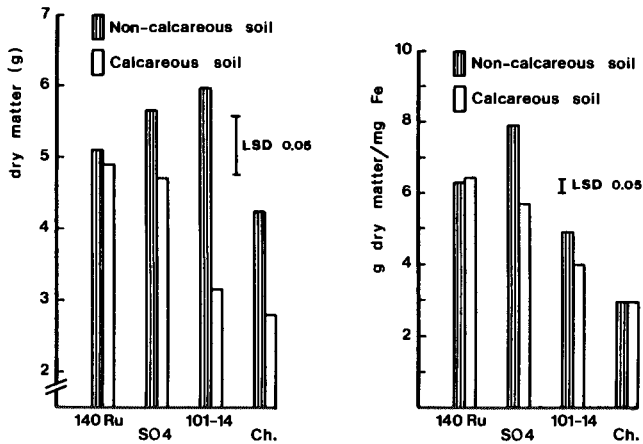


Fig. 5 (left): Dry matter production (average value of the four organs) depending on genotype and soil at the end of the annual growing period.

Fig. 6 (right): Iron efficiency ratio (g dry matter/mg Fe) in the shoot depending on genotype and soil.

Despite the lack of chlorosis symptoms, 101-14 rootstock grown on calcareous soil differs from the other two rootstocks (more resistant to lime-induced chlorosis) by having the lowest shoot growth, dry matter production (at the end of the growing period) and 'iron efficiency ratio' (g dry matter/mg Fe) of the shoot. A difference in the dry matter production between susceptible (3309) and resistant (Fercal, 140 Ru) rootstocks was also observed by CHIADMI and BRANCHARD (1987) in *in vitro* trials.

On the other hand, 140 Ru rootstock shows its characteristics of resistance by having in calcareous soil the highest shoot growth, dry matter production and 'iron efficiency ratio' of the shoot; moreover, it does not change its behaviour depending on the soil.

SO4 rootstock has characteristics intermediate between 101-14 and 140 Ru. Chardonnay (which is normally grown grafted) seems a genotype with high iron requirements, but low 'iron efficiency ratio'.

Conclusions

The most significant findings are that:

1. during the growing period, the chlorophylls and leaf Fe(II) content first increases and then decreases;
2. the rootstock most susceptible to chlorosis (101-14) shows in the calcareous soil the highest Fe(II) and total chlorophyll content, while 140 Ru rootstock (the most resistant) has the lowest values;
3. suitable tools to judge the resistance/susceptibility to lime-induced chlorosis in ungrafted rootstocks grown on calcareous soil are: a) the dry matter production at the end of the annual growing period; b) the 'iron efficiency ratio' (g dry matter/mg iron) in the shoot at the end of the annual growing period.

Acknowledgements

The author wants to thank Mr. GIUSEPPE BRUZZI (laboratory staff) for his contribution to this project.

References

- BALASUBRAHMANYAM, V. R.; EIFERT, J.; DIOFASI, L.; 1978: Nutrient reserves in grapevine canes as influenced by cropping levels. *Vitis* 17, 23-29.
- BAVARESCO, L.; FREGONI, M.; FRASCHINI, P.; 1989: Investigations on iron uptake and reduction by excised roots of different grapevine rootstocks and a *V. vinifera* cultivar. 5th Intern. Symp. Iron Nutrition and Interaction in Plants, Kibbutz Ramat-Rachel, Israel, 11-17 June 1989.
- BIENFAIT, H. F.; DUIVENVOORDEN, J.; VERKERKE, W.; 1982: Ferric reduction by roots of chlorotic bean plants: indications for an enzymatic process. *J. Plant Nutr.* 5, 451-456.
- BOXMA, R.; 1972: Bicarbonate as the most important soil factor in lime-induced chlorosis in the Netherlands. *Plant Soil* 37, 233-243.
- BROWN, J. C.; JONES, W. E.; 1976: A technique to determine iron efficiency in plants. *Soil Sci. Soc. Amer. J.* 40, 398-405.
- BYRNE, D. H.; 1988: Comparative growth of two peach seedling rootstocks under alkaline soil conditions. *J. Plant Nutr.* 11, 1663-1669.
- CHEN, Y.; BARAK, P.; 1982: Iron nutrition of plants in calcareous soils. *Adv. Agron.* 35, 217-240.
- CHIADMI, N.; BRANCHARD, M.; 1987: Mise au point d'un test 'in vitro' permettant la détermination précoce de la sensibilité de porte-greffes de vigne à la chlorose calcaire. 3e Symp. Intern. Physiol. Vigne, Bordeaux, 42-44.
- COTTENIE, A.; 1980: Soil and plant testing as a base of fertilizer recommendation. *FAO Soils Bull.* 38 (2).
- COULOMBE, B. A.; CHANEY, R. L.; WIEBOLD, W. J.; 1984: Bicarbonate directly induces iron-chlorosis in susceptible soybean cultivars. *Soil Sci. Soc. Amer. J.* 48, 1297-1301.
- FREGONI, M.; SCIENZA, A.; 1975: Ricerche sull'assimilabilità del ferro in vigneti italiani. *Vignevini* 6, 7-10.
- HETHER, N. H.; OLSEN, R. A.; JACKSON, L. L.; 1984: Chemical identification of iron reductants exuded by plant roots. *J. Plant Nutr.* 7, 667-676.
- JUSTE, C.; POUGET, R.; BRUZAU, F.; 1967: Influence du pH et de l'anion bicarbonique sur l'absorption du fer par les racines de vigne. *C. R. Acad. Sci.* 264, 2781-2784.
- KATYAL, J. C.; SHARMA, B. D.; 1980: A new technique of plant analysis to resolve iron chlorosis. *Plant Soil* 55, 105-119.
- KOLESCH, H.; HOFNER, W.; SCHALLER, K.; 1987: Effect of bicarbonate and phosphate on iron chlorosis of grape vines with special regard to the susceptibility of two rootstocks. Part. II: pot experiments. *J. Plant Nutr.* 10, 231-249.
- KRAMER, D.; RÖMHELD, V.; LANDSBERG, E.; MARSCHNER, H.; 1980: Induction of transfer-cell formation by iron deficiency in the root epidermis of *Helianthus annuus* L. *Planta* 147, 335-339.
- LANDSBERG, E. C.; 1981: Organic acid synthesis and release of hydrogen ions in response to Fe-deficiency stress of mono- and dicotyledonous plant species. *J. Plant Nutr.* 3, 579-591.
- ; 1989: Proton efflux and transfer cell formation as response to Fe-deficiency of soybean in nutrient solution culture. *Plant Soil* 114, 53-61.
- MARSCHNER, H.; 1978: Beziehung zwischen der Eisenversorgung von Weinreben und dem pH-Verlauf in der Nährlösung. *Vitis* 17, 152-160.
- ; 1986: Mineral Nutrition in Higher Plants. Academic Press, London.
- ; RÖMHELD, V.; KISSEL, M.; 1986: Different strategies in higher plants in mobilization and uptake of iron. *J. Plant Nutr.* 9, 695-713.
- MENGEL, K.; BÜBL, W.; 1983: Verteilung von Eisen in Blättern von Weinreben mit HCO₃⁻ induzierter Fe-Chlorose. *Z. Pflanzenernähr. Bodenk.* 146, 560-571.
- ; BREININGER, M. TH.; BÜBL, W.; 1984: Bicarbonate the most important factor inducing iron chlorosis in vine grapes on calcareous soil. *Plant Soil* 81, 333-344.
- ; GEURTZEN, G.; 1986: Iron chlorosis on calcareous soils. Alkaline nutritional condition as the cause for the chlorosis. *J. Plant Nutr.* 9, 161-173.
- ; MALISSIOVAS, N.; 1981: Bicarbonat als auslösender Faktor der Eisenchlorose bei der Weinrebe (*Vitis vinifera*). *Vitis* 20, 235-243.
- MURISIER, F.; BRIGUET, C.; 1988: Rendement et chlorose de la vigne. *Rev. Suisse Vitic. Arboric. Hortic.* 20, 165-172.

- NERKAR, Y. S.; MISAL, M. B.; 1987: Rice breeding for tolerance to iron chlorosis. *J. Maharashtra Agricult. Univ.* **12** (3), 385-386.
- POUGET, R.; 1974: Influence des réserves glucidiques sur l'intensité de la chlorose ferrique chez la vigne. *Conn. Vigne Vin* **8**, 305-314.
- ; 1980: Breeding grapevine rootstocks for resistance to iron chlorosis. *Proc. 3rd Intern. Symp. Grape Breeding*, Davis, California, 191-197.
- ; JUSTE, C.; 1972: Le choix des porte-greffes de la vigne pour les sols calcaires. *Conn. Vigne Vin* **6**, 357-364.
- ; OTTENWALTER, M.; 1973: Etude méthodologique de la résistance à la chlorose calcaire chez la vigne: principe de la méthode des greffages réciproques et application à la recherche de porte-greffes résistants. *Ann. Amélior. Plantes* **23**, 347-356.
- ROMERA, F. J.; ALCANTARA, E.; DE LA GUARDIA, M. D.; 1989 a: Characterization of the tolerance to iron chlorosis in different peach rootstocks grown in nutrient solution. I. Effect of bicarbonate. *5th Intern. Symp. Iron Nutrition and Interaction in Plants*, Kibbutz Ramat-Rachel, Israel, 11-17 June 1989.
- ; -- ; -- ; 1989 b: Characterization of the tolerance to iron chlorosis in different peach rootstocks grown in nutrient solution. II. Response mechanisms. *5th Intern. Symp. Iron Nutrition and Interaction in Plants*, Kibbutz Ramat-Rachel, Israel, 11-17 June 1989.
- RÖMHELD, V.; 1987: Different strategies for iron acquisition in higher plants. *Physiol. Plant.* **70**, 231-234.
- ; MARSCHNER, H.; 1981: Iron deficiency stress induced morphological and physiological changes in root tips of sunflower. *Physiol. Plant* **53**, 354-360.
- ; -- ; 1986: Evidence for a specific uptake system for iron phytosiderophores in roots of grasses. *Plant Physiol.* **80**, 175-180.
- STRAIN, H. H.; SVEC, W. A.; 1966: Extraction, separation, estimation, and isolation of the chlorophylls. In: VERNON, L. P.; SEELY, G. R. (Eds.): *The Chlorophylls*, 21-66. Academic Press, London.
- TAKAGI, S.; NOMOTO, K.; TAKEMOTO, T.; 1984: Physiological aspect of mugineic acid, a possible phytosiderophore of graminaceous plants. *J. Plant Nutr.* **7**, 469-477.
- TORRECILLAS, A.; LEON, A.; DEL AMOR, F.; MARTINEZ-MOMPEAN, M. C.; 1984: Determinación rápida de clorofila en discos foliares de limonero. *Fruits* **39**, 617-622.